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PRELIMINARY ANTIMICROBIAL SCREENING OF SIXTY INDIGENOUS PLANTS FROM KHANDESH REGION, MAHARASHTRA

S.S. PATOLE AND R.T. MAHAJAN*
DEPARTMENT OF ZOOLOGY V.V.M'S.S.G. PATIL ARTS, SCIENCE AND COMMERCE COLLEGE, SAKRI-424 304, INDIA.
PG-DEPARTMENT OF ZOOLOGY, MOOLJI JAITHA COLLEGE, JALGAON-425 001, INDIA*.
(e-mail : vidyavikas2006@rediffmail.com)

Total aqueous extract (TAE) of 60 indigenous plant species were screened for their *in vitro* antimicrobial activity against two bacterial species *i.e. Bacillus subtillis* and *Escherichia coli* and single fungus *i.e. Aspergillus nigar*. The results from well diffusion method showed that of the 60 plants, 27 plants inhibited the growth of *Bacillus subtillis*, 14 plants against *Escherichia coli* and 19 plant extracts acted as antifungal against *Aspergillus nigar*. 11 plants showed a broad spectrum of antimicrobial activity whereas 32 plant extracts exhibited negligible activity. The zone of inhibition of the TAE of plants was ranging from 5 to 15 mm. The results indicate that *Allium sativum Linn, Azadirachta indica A. Juss, Curcuma longa* Linn and *Tridex procumbens* Linn possesses potential broad spectrum antimicrobial activity.

Key words: Antimicrobial, indigenous plants, *Bacillus subtillis, Escherichia coli, Aspergillus nigar,*

INTRODUCTION

Nature in its own complex has endowed many medicinal plants having ability to synthesize a wide variety of chemical substances; which when applied by man are known to produce a physiological change in normal biological system. These natural plant products are fast catching attention of scientists for their use as biopesticides to control insect pest as well as antimicrobial infestations (Verma & Dubey, 1999; Patil *et al.*, 2002; Mahulikar *et al.*, 2005; Kubmarawa *et al.*, 2007). Traditional medicines having raw and processed food have been used as alternative medicines for a wide variety of purposes for many years in all over world. However, most of the applications have not been evaluated scientifically, and their effects have not been explained experimentally yet (Benli, 2007). About 25-30 % of the medicines used for people's health have been obtained from indigenous plants (Raju *et. al.*, 2004; Karuppusamy & Karmegam, 2005; Ignacimuthu *et al.*, 2006).

Jalgaon, Nandurbar and Dhule districts (Khandesh region) of North Maharasht. have a huge flora and broad tradition of medicinal plants (Patil, 2003; Mali *et al.*, 2006). Unfortunately information regarding the use of medicinal plants are being unknown due to freezing of ancient information about mode of use. On other hand long term use of antibiotics against microbial infections is out dated because of exacerbated antibiotic resistance (Rawat & Uniyal, 2003; Kumar *et al.*, 2007). The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatment for microbial diseases (Leven *et al.*, 1979; Samy & Ignacimuthu, 2000; Gomathi & Suriyavathana, 2005; Kubmarawa *et al.*, 2007). In present studies, we report the *in vitro* effect of Total aqueous extracts of 60 indigenous plants against common pathogens like *Bacillus subtillis*, *Escherichia coli* and *Aspergillus*

nigar which are common infectious agents, particularly skin and mucosal infections are common in a wide range of hosts. It is also main aim of this paper to investigate the locally available indigenous plant that will acts as common antimicrobial remedy.

MATERIALS AND METHODS

Plant material : Plants used in present study were collected during February to May 2007 at Sakri (M.S.) and near by places. All the plants were identified at Botany department of this College where their voucher specimens are deposited (Table I).

Preparation of plant extract : Total aqueous extracts (TAE) were prepared by soxhlet method. The plant material were dried under shade and ground into fine powder using electric blender. A sample (30 g) of each powdered plant material was individually soxhlet extracted in 300 ml of water at 95^{0}C for 6 h. At the end of the extraction, each extract was concentrated in vacuum evaporator at 30^{0}C and stored at 4^{0}C until further use. For antimicrobial screening, 1% TAE was prepared freshly before use.

Test organisms : TAE of 60 indigenous plants were screened against two bacterial strains, namely *Bacillus subtillis* and *Escherichia coli* and one fungal strain, *Aspergillus nigar*. The test organisms were obtained from the Department of Microbiology, Moolji Jaitha College, Jalgaon. The cultures were maintained at 4^{0}C on slope of nutrient agar (for bacteria species) and dextrose agar (for fungus) obtained from Himedia (Mumbai). 1% inoculums were prepared using fresh culture media.

Antimicrobial screening : For antimicrobial screening activity of the plant extracts against bacteria and fungi species, agar well diffusion biological assay technique (Deena *et al.*, 2002) was employed. To each autoclaved petri plate (100 mm diameter), approximately 20 ml of sterilized medium was added. Allow few minutes to set the medium. To medium, 2-3 drops of inoculum (1% suspension culture) was added to each petri plates and spread thoroughly by glass spreader. After inoculation, cups were scooped out with 6 mm sterile cork borer and to each cup; TAE of plants was added aseptically with the help of sterile micropipette. For facilitating diffusion, the plates were kept in refrigerator for 15 minutes. Nutrient agar (Bacterial) petri plates were kept in incubator at 37^{0}C for 24 h. whereas dextrose agar (Fungal) petri plates was incubated at room temperature maximum up to 120 h. At end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimeter. A standard antibiotic, Gentamicin (30 µg/well) was used for comparison of antibacterial activity of plant extracts. Controls were maintained with water only. Three replicas were done for each treatment.

RESULTS AND DISCUSSION

The screening result of TAE of sixty indigenous plants against two bacterial and one fungus species is summarized in Table I. As per this table, among 60 plants tested, 27 plants showed activity against *B. subtillis*, 14 plants against *E. coli* and 19 plants acted as antifungal against *A. nigar*. Beside this 11 plants acted strongly both antibacterial and antifungal activity. From same Table it is seen that, the TAE of *Tridax procumbens*, *Allium sativum*, *Azadirachta indica*, *Curcuma longa*, *Mentha arvensis and Sphaeranthus indicus* showed higher zone of inhibition in both bacterial and fungus species. Whereas TAE of other plants *viz.* *Adhatoda vasica*, *Annona squamosa*, *Euphorbia tirucalli*.

Table 1 : Antimicrobial activity of TAE of indigenous plants.

Botanical name	Antimicrobial activity		
	B. subtillis	***E. coli***	***A. nigar***
Acacia concinna D.C	-	-	-
Acorus calamus Linn	+	-	+
Adhatoda vasica Ness	+	+	+
Agave Americana Linn	-	-	-
Allium sativum Linn	+++	++	+
Anagallis arvensis Linn	-	-	-
Anamirta cocculus Linn	-	-	-
Andropogon schoenthus Linn	+	+	+
Annona muricata Linn	-	-	-
Annona squamosa Linn	++	+	+
Azadirachta indica A. Juss	+++	++	+
Balanites roxburghiii Planch	+	+	-
Barringtenia racemosa Linn	-	-	-
Brassica nigra Linn	-	-	-
Butea monosperma Linn	-	-	-
Capsicum annum Linn	-	-	-
Citrullus colocynthis Scharad	+	-	-
Clerodendrum phlomidis Linn F.	-	-	-
C. serratum	-	-	-
Cocculus hirsutus Linn	-	-	-
Coriandrum sativum Linn	-	-	-
Croton tiglium Linn	-	-	-
Curcuma longa Linn	+++	++	+
Datura metal Linn	-	-	+
Dodonaea viscose Linn	-	-	-
Duranta repens Linn	+	-	+
Echinops echinatus Roxb.	-	-	-
Eucalyptus globule Labill	+	-	+
Euphorbia tirucalli Linn	++	-	-
Gardenia lucida Roxb.	-	-	-
Gloriosa superba Linn	-	-	-
Jatropha curcas Linn	-	-	-
Lantana camera Linn	++	+	+
Lycopersicon esculentum Mill	-	-	-
Madhuca indica Roxb	+	-	-
Melia azadirachta Linn	++	+	+
Mentha arvensis Linn	++	+	+
Murraya koenigii Spreng	+	-	-
Nerium oleander Linn	-	-	-
Nicotiana tabacum Linn	-	-	-
Ocimum sanctum Linn	++	+	+
Parthenium hysterophorus Linn	-	-	-
Peganum harmala Linn	-	-	-
Phyllanthus niruri Linn	-	-	-
Polygonum hydropiper Linn	-	-	-
Pongamia glabra Linn	++	-	+
Randia dumetorum Lamk	-	-	-

Ricinus communis Linn	-	-	-
Sapindus trifoliatus Linn	-	-	-
Sphaeranthus indicus Linn	++	+	+
Spilanthes acmella Murr	-	-	-
Tagetes minuata Linn	+	-	+
Tephrosia purpurea Pers	++	-	-
Thevetia nerifolia Juss	-	-	-
Trigonella foenum graecum Linn	-	-	-
Tridax procumbens Linn	+++	+++	-
Vinca rosea Linn	+	-	+
Vitex negundo Linn	+	+	+
Withania somnifera Dunal	+	-	-
Zingiber officinale Rose	+	-	-
Gentamicin (Std. Antibiotics)	+++	+++	-
Untreated Control	-	-	-

+++= Zone of inhibition (13 mm on words) ++ = Zone of inhibition (9-12 mm)
+ = Zpme pf omjobotopm (5-8 mm) - = No activity

Lantana camera, Melia azadirachta, Ocimum sanctum and *Vitex nigundo* showed moderate antimicrobial activity in test organisms. The antimicrobial activity of some of these plants has been studied previously. Chary *et al.* (1984) revealed, the antifungal principles of aqueous extract of *Azadirachta indica* A. Juss, *Jatropha curcus* Linn., *Melia azadirachta* Linn., *Thevetia nerifolia* A. Juss, *Trigonella foenum graecum* Linn. and *Vinca rosea* Murr. All these plants were reported the per cent of spore germination inhibition in two fungi species. Mahajan *et al.* (1999) revealed the screening of *Balanites roxburghii*, *Tephrosia purpurea* and *Sphaeranthus indicus* for antibacterial activity. They reported that the antibacterial principles are highly distributed in *Sphaeranthus indicus*, moderately in *Balanites roxburghii* and very less in *Tephrosia purpurea*. Biological activity of n-butane extract of *Tridax procumbens* against some gram positive, gram negative, yeast and fungi were reported by Taddei & Romero (2000), the flower extract showed activity against *Escherichia coli* whereas whole aerial part extract active against *Mycobacterium smegmatis*, and *Salmonella typhi*. Dubey *et al.* (2000) reported crude extract of *Sphaeranthus indicus* is highly effective against *Altenaria solani*, *Fusarium oxysporum* and *Penicillium pinophilum* species. Patil *et al.* (2002) showed spectacular antibacterial & and antifungal activity of solvent extracts of *Duranta repens*. Gomathi & Suriyavathana (2005) revealed antifungal activity of *Ocimum sanctum* against *Aspergillus nigar*. Kumar *et al.* (2007) evaluated antimicrobial activity of some indian medicinal plants against various species of acne-inducing bacteria. The present findings of TAE against common skin disease inducing microbes are in agreement with previous workers.

Intensive use of antibiotics often resulted in the development of resistance strains (Sydney *et al.*, 1980; Verma & Dubey, 1999; Rawat & Uniyal, 2003). Hence, the search for new antibiotics continues unbated. In this connection plants continue to be a rich source of therapeutic drugs. India has a great variety of plants used in folk medicine and only a few of these have been studied for their antimicrobial studies. The results obtained from present investigation indicated the existence of antimicrobial compounds in the TAE of these plants and some showed good correlation between reported uses of these plants in traditional medicines against infectious diseases for example the inhibition of *E.*

coli by the extract of *Tridex, Azadirachta, Sphaeranthus* etc has justified their use for treatment of skin diseases like eczema, scabies and others in the traditional medicines.

Natural plant products are fast caching attention of scientist for their use as antimicrobial to control bacterial and fungal diseases. Unlike synthetic pesticides they do not cause pollution and undesired side effects on environment. It is very likely that the current global thrust on herbal pesticides may yield, lead and prototype molecules to guide future research programme in pest management. Some of these plant extracts have antibacterial activity, and the activities eradicate the bacteria completely. Effective compounds to be obtained by the determination of the active compounds in the plant can provide new resources for chemotherapeutics to be synthesized. It will be a base to our future investigation on advance purification.

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REFERENCES

BENLI, M., GUNEY, K., BINGOL, U., GEVEN, F. & YIGIT, N. 2007. Antimicrobial activity of some endemic plant species from Turkey. *African J Biotech.* **6**(15) : 1774-1778.

CHARY, M.P., REDDY, E. J. S. & REDDY, S.M. 1984. Screening of Indigenous plants for their antifungal principles. *Pesticides.* **8** : 17-18.

DEENA, M.J., SREERANJINI, K. & THOPPIL, I.E. 2002. Antimicrobial screening of essential oils of *Coleus aromaticus* and *Coleus zeylanicus. Indian. J Aromatherapy* **12** : 105-107.

DUBEY, K.S., ANSARI, A.H. & HARDAHA, M. 2000. Antibacterial activity of the extract of *Sphaeranthus indicus. Asian J Chem.* **12**(2) : 577-578.

GOMATHI, M.S. & SURIYAVATHANA, M. 2005. Screening for antifungal activity of selected medicinal plants. *J Ecobiol.* **17**(5) : 445-449.

IGNACIMUTHU, S., DURAIPANDIYAN, V. & AAYYANAR, M. 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary* and *Alternative Medicine.* **6**(35) : 1-8.

KUBMARAWA, D., AJOKU, G.A., ENWEREM, N.M. & OKORIE, D.A. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African J Biotech.* **6**(14) : 1690-1696.

KUMAR, G.S., JAYAVEERA, K.N., ASHOK KUMAR, C.K., SANJAY, U.P., SWAMY, B.M.V. & KUMAR, D.V.K. 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. *Trop. J. Pharma. Res.* **6**(2): 717-723.

KARUPPUSAMY, S. & KARMEGAM, N. 2005. Screening of ethnomedicinal plants of Dindigul district (South India) for antimicrobial activity. *J. Ecobiol.* **17**(5) : 455-459.

LEVEN, M., DIRK, A., VENDEN BERGHE, V., FRANCIS, M., VLIETINCK, A. & LAMMENS, E. 1979. Screening of higher plants for biological activities-I, Antimicrobial activity. *Planta Medica.* **36** : 311- 321.

MAHAJAN, R.T., CHAUDHARI. G.S. & CHOPDA, M.Z. 1999. Screening of some indigenous plants for their possible Antibacterial activity. *Environ. Bull.* **15** : 61-62.

MAHULIKAR, P.P., SHIMPI, S.R., CHAUDHARI, L.S., BHARAMBE, S.M., KHARCHE, A.T., PATIL, K.P. & BENDRE, R.S. 2005. Evaluation of antimicrobial activity of organic extract of leaves of *Aristolochia brasteata. Pesticide Res. J.* **17**(1) : 16-18.

MALI, R.G., HUNDIWALE, J.C., GAVIT, R.S., PATIL, D.A. & PATIL, K.S. 2006. Herbal abortifacients used in North Maharashtra. *Nat. Prod Radience* **5**(4): 315-318.

PATIL, V.J., DESHMUKH, M.B. & MANER, M.I. 2002. Antimicrobial and Antifeedant activity of the extract of the plant *Duranta repens*. *Biotech. Agric. Industry and Environ.* Microbiologist Society, Karad. pp. 65-67.

PATIL, D.A. 2003. *Flora of Dhule and Nandurbar Districts*. (Maharashtra). Bishen Singh Mahendra Pal Singh, Dehra Dun-248001 (India).

RAJU, V.R.R., RAM, A.J. & BHAKSHU, M.L. 2004. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. *J. Ethanopharmacology* **90** : 353-357.

RAWAT, R.B.S. & UNIYAL, R.C. 2003. National Medicinal Plants Board Committed for overall development of the Sector. *Agro. Bios. Med Plants*. **1** : 12-16.

SAMY, R.P. & IGNACIMUTHU, S. 2000. Antibacterial action of some folklore medicinal plants used by tribals of Western Ghats of *India. J Ethnopharmacol.* **69**(1) 63-71.

SYDNEY, S., LACY, R.W. & BAKHTIAR, M. 1980. *The Betalactam antibiotics Penicillin and Cephalosporin in perspective.* Hodder and stongton, London, pp. 224.

TADDEI, A & ROSAS-ROMERO, A.I. 2000. Bioactivity studies of extracts from *Tridax procumbens. Phytomedicines*. 7(3) : 235-238.

VERMA, J. & DUBEY, N. 1999. Prospective of botanical and microbial products as pesticides of tomorrow. *Curro Sci.* **76** :172-179.